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DEVELOPMENT AND VALIDATION OF UVSPECTROPOTOMETRIC METHODS FOR QUANTITATIVE ESTIMATION OF TENOFOVIR DISPROXIL FUMARATE IN BULK & PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Zero-order and Area Under Curve [AUC] UV-Spectrophotometric methods have been developed and validated for the estimation of Tenofovir disproxil fumarate in bulk and its tablet formulations. The solutions of standard and sample were prepared in water. Tenofovir disproxil fumarate was estimated at 260 nm and for the zero order UV-Spectrophotometric methods, respectively, while area under curve for the zero order spectrum of Tenofovir disproxil fumarate between 250 nm to 270 nm was measured for AUC method. Beer's law was obeyed in the concentration range of 5 - 30 µg / ml with r^2 value 0.999 for zero order. In AUC method, beer's law was obeyed in the concentration range of 5-30 µg / ml with r^2 value 0.999. These methods were tested and validated for various parameters according to ICH guidelines. The proposed methods were successfully applied for the determination of Tenofovir disproxil fumarate in tablet formulations.

Keywords: Tenofovir disproxil fumarate, UV-Spectrophotometric methods, beer's law.

INTRODUCTION

Tenofovir disoproxil fumarate, marketed by Gilead Sciences under the trade name Viread, belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (NRTIs), which block reverse transcriptase, an enzyme crucial to viral production. Tenofovir disoproxil fumarate is a prodrug form of tenofovir. Tenofovir is also available in a fixed-dose combination with emtricitabine in a product with the brand name Truvada for once-a-day dosing Atripla [1,2].

Objectives

The target of this study is to develop new, simple and fast analytical methods by UV Spectrophotometry

[3,4] to quantify Tenofovir disoproxil fumarate in bulk and its tablet dosage forms together with its latter validation study. The target of this study is to develop new, simple and fast analytical methods by UV Spectrophotometry. This validation study is defined as the laboratory studies by which it is established that the performance characteristics of the method meet requirements for the intended analytical application. This work describes the validation parameters stated by the International Conference on Harmonization [ICH] guidelines includes specificity, linearity, range, accuracy, precision, robustness to achieve analytical methods with acceptable characteristics of suitability, reliability and feasibility.

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EXPERIMENTAL WORK

Materials

Spectral runs were made on a Shimadzu UV Visible spectrophotometer, model-1800 (Japan) with spectral bandwidth of 0.5 nm, wavelength accuracy of 0.3 nm with automatic wavelength corrections using a pair of 10 mm quartz cells. All Spectral measurements were done using UV-Probe 2.33 software. Glassware's used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven. Tenofovir disoproxil fumarate reference standard was kindly provided by Sushan pharma limited, pandichery. Commercial tablet formulations were used for present study containing 245mg of Tenofovir disoproxil fumarate, respectively. Water was used as solvent for dilutions. All the solutions were analyzed on the day of preparations.

Preparation of working standard drug solution

The standard Tenofovir disoproxil fumarate (100 mg) was weighed accurately and transferred to volumetric flask (100 ml). It was dissolved properly and diluted up to the mark with water to obtain final concentration of 1000 µg/ml and the resulting solution was used as working standard solution.

Analysis of marketed formulations

For the estimation of Tenofovir disoproxil fumarate in tablets formulation, 20 tablets were weighed and triturate to fine powder. Tablet powder equivalent to 100 mg of Tenofovir disoproxil fumarate for each was weighed and transfer into 100 ml volumetric flask than dissolved with water and further diluted with water. It was kept for ultra-sonication for 30 min; this was filtered through Whatman filter paper No. 41 and then final dilution was made with water to get the final stock solution of 1000 µg/ml. From this stock solution, various dilutions of the sample solution were prepared and analysed.

Zero order spectrophotometry

The solutions were scanned in the range 400- 200 nm using water as blank to obtain zero order UV-Spectra of Tenofovir disoproxil fumarate (Fig. 2). The peak was observed at 260 nm which was used as an analytical wavelength for measurement of absorbance. Calibration curve was obtained by plotting absorbance at 260nm against the concentration of Tenofovir disoproxil fumarate. The regression equation and correlation coefficient were determined. Beer's- Lamberts law was obeyed in the concentration range of 5-30 µg/ml. The concentration of the sample solution was determined using the regression equation.

AUC spectrophotometry

In this method, Zero order UV-Spectra were obtained and Area Under the Curve [AUC] between the

range 250-270 nm were measured using UV-Probe as illustrated in Fig. 3 Calibration curve was obtained by plotting AUC between 250-270 nm against the concentration of Tenofovir disoproxil fumarate. The regression equation and correlation coefficient were determined. Beer's- Lamberts law was obeyed in the concentration range of 5-30 µg/ml. The concentration of the sample solution was determined using the regression equation.

Validation

The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines [5,6,7] Q2A and Q2B. Recommend validation characteristics depend on the type of analytical procedure. Method validation characteristics were tested in accordance with ICH guidelines for each method. Linearity (correlation coefficient) was tested in the given range for each method. Repeatability and Intermediate precisions were obtained as % Relative Standard Deviation [% RSD] using six replicates per day. We have established method accuracy (% Recovery and SD) by spiked placebo recovery method. Limits of detection and quantification were provided for Tenofovir disoproxil fumarate using standard deviation of intercept. To establish ruggedness of the proposed methods, assays for two different brands of Tenofovir disoproxil fumarate tablets were performed by two different analysts on two different days.

RESULTS AND DISCUSSION

Tenofovir disoproxil fumarate has the absorbance maxima at 260 nm and Zero order derivative spectra, showed sharp peak at 260 nm. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 5-30 µg/ml and given in Table 1. Recovery studies were carried out at three different levels i.e. 50 %, 100 %, and 150 % by adding the pure drug to the previously analysed tablet powder sample. Percentage recovery for Tenofovir disoproxil fumarate was determined by all the methods and they were found to be under acceptance criteria which are 98% to 102 % according to ICH guidelines. The results are in Table 2. The percentage recovery value indicates non interferon from excipients used in formulation. The result of analysis of marketed formulation is shown in Table 2. The reproducibility and accuracy of the method was found to be good, which was evidenced by low standard deviation.

Figure :1 Chemical structure of Tenofovir disoproxil fumarate

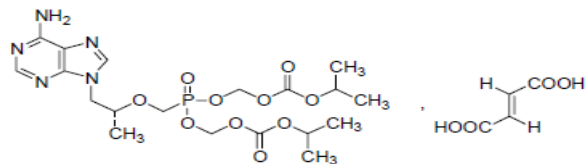


Figure 2. Zero order spectra of Tenofovir disproxil fumarate showing absorbance Area Under Curve [AUC] from 250 to 270 nm.

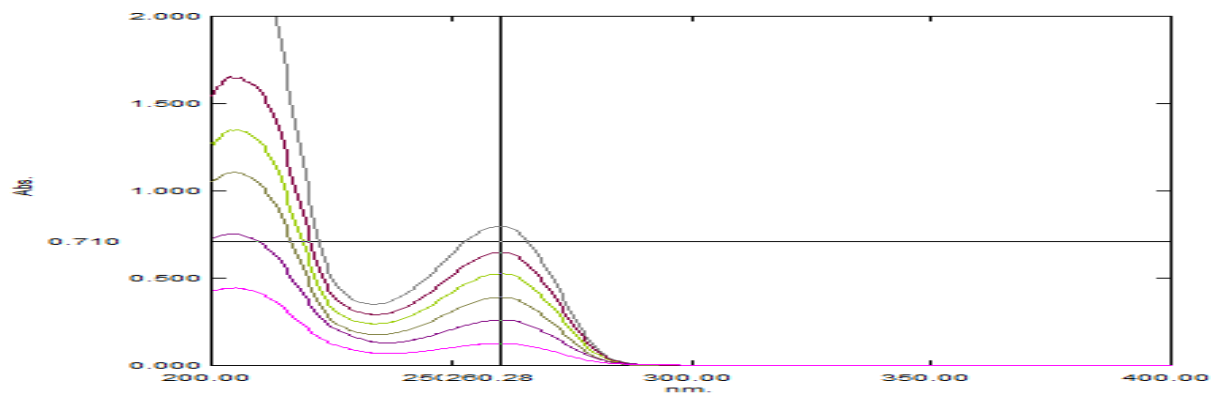


Figure 3. Zero order spectra of Tenofovir disproxil fumarate showing Area Under Curve [AUC] from 250 to 270 nm.

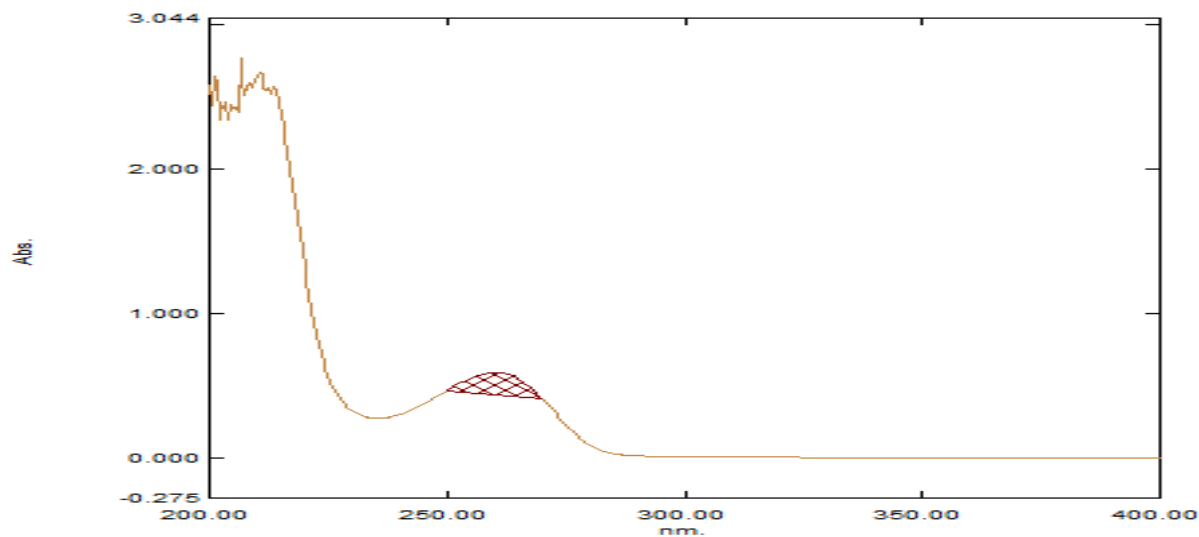


Table 1. Validation parameters

Validation Parameters	Zero-order method	AUC method
Absorption Maxima (nm)	260	250-270
Beer's-Lambert's range ($\mu\text{g}/\text{ml}$)	5-30	5-30
Regression equation (y)*		
Slope (b)	0.025	0.078
Intercept (a)	0.000	-0.004
Correlation coefficient	0.999	0.999
Sandell's sensitivity ($\text{mcg} / \text{cm}^2 \cdot 0.001$ absorbance units)	0.038536	0.012796
Precision (% RSD)		
Intraday precision	0.157523	0.052261

Interday precision	0.172669	0.057262
Accuracy	99.08	99.087
Limit of detection ($\mu\text{g} / \text{ml}$)	0.06312	0.02074
Limit of quantification ($\mu\text{g} / \text{ml}$)	0.191277	0.06286

* Mean \bar{x} \pm SD are obtained from 6 determinations.

Table 2. Analysis of formulations.

Tablet	Label claimed (mg)	Amount found (mg)		%Recovery \pm SD**	
		Zero-order method	AUC method	Zero-order method	AUC method
Viread	245mg	242.77	242.76	99.08 \pm 0.48	99.05 \pm 0.45

* Obtained from 6 determinations

CONCLUSION

The proposed analytical methods are rapid, accurate, precise and reproducible and hence can be used for the routine analysis of Tenofovir disoproxil fumarate in bulk, tablet dosage forms. The sample recoveries from the formulation were in good agreement with their respective label claims, which suggested non-interference of excipients in the estimation. The most striking features of

these methods are its simplicity and rapidity, not requiring tedious sample preparations such as extraction of solvents, heating, degassing which are may needed for HPLC procedure. All the above result indicates that, the methods employed here are very simple, accurate, economic and rapid for routine analysis of the Tenofovir disoproxil fumarate.

REFERENCES

1. Anonymous 1. URL:http://en.wikipedia.org/wiki/Tenofovir_disoproxil_fumarate
2. Anonymous 2. URL:<http://www.rxlist.com/vimpat-drug.htm>
3. Shirkhedkar Atul A, Bhirud Charushila H and Surana Sanjaja. Application UV- spectrophotometric methods for estimation of tenofovir disoproxil fumarate in tablets. *Pak. J. Pharm. Sci.*, 22(1), 2009, 27-29
4. Anindita Behera, Aurobinda Parida, Amit Kumar Meher, Dannana Gowri Sankar, Swapan Kumar Moitra, Sudam Chandra SI. Spectrophotometric method for Development and Validation of determination of Emtricitabine and Tenofovir Disoproxil Fumarate in Bulk and Tablet dosage form. *Int.J. PharmTech.*, 3(3), 2011, 1878-1882.
5. ICH. Note for guidance on validation of analytical methods: Definitions and Terminology Q2A; 1994.
6. ICH. Note for guidance on validation of analytical procedures: Methodology Q2B; 1996.
7. ICH, Q2 (R1) Validation of Analytical Procedures: text and methodology; 2005.